Microcirculation of the Pancreas in the Rat and Rabbit with Special Reference to the Insulo-Acinar Portal System and Emissary Vein of the Islet

Osamu OHTANI, Tatsuo USHIKI, Hiroaki KANAZAWA and Tsuneo FUJITA

Department of Anatomy (Prof. T. MURAKAMI), Okayama University Medical School, Okayama, and Department of Anatomy (Prof. T. FUJITA), Niigata University Medical School, Niigata, Japan

Received December 28, 1985

Summary. Microcirculation of the pancreas in the rat and rabbit with special reference to the islets was studied by scanning electron microscopy (SEM) of vascular corrosion casts, light microscopy (LM) of India ink-injected/cleared tissues, and intravital microscopy of in situ organs. The following observations were made:

1) Approximately 10-20% of the total terminal arterioles supplied the islets, while the remainder directly supplied the exocrine pancreas.
2) The vas afferens of the islets divided into sinusoidal capillaries with frequent U-shaped turns in the cortical A and D cell area of the islets, and their secondary branches supplied the core B cell area. Intravital microscopy confirmed that blood irrigated the cortex of the islets first and the core portion second.
3) All islets observed possessed insulo-acinar portal vessels.
4) About 60% of the islets in the rat possessed emissary veins leading directly into the systemic circulation, while in the rabbit, less than 5% of islets possessed emissary venules of small diameter. Thus, the well-developed emissary veins of the islets seemed characteristic of the rat, as compared with the rabbit and several other mammals examined previously.
5) The insulo-acinar portal system seems to represent a short vascular route through which islet secretions are transported in high concentrations to the exocrine pancreas, there to exert their actions. The emissary veins of the islet seem to serve for the quick conveyance of insular secretions into general circulation.
6) It is suggested that the pancreatic lobule is made up of subdivisions or microcirculatory units, each of which is supplied centrally by the insulo-acinar portal system, while peripherally the unit also receives direct branches of intralobular arterioles. The veins run the periphery of the unit.
7) The occurrence of sphincters in the vas afferens and the emissary veins of the islets is suggested as being involved in the regulation of the islet blood flow.

"Why are the islets of Langerhans in the pancreas?" is a question that has attracted many researchers' interest. Some authors (e.g., HENDERSON, 1969; FUJITA, 1973) have thought that a clue to the answer lies in the vascular connections between the islets and the exocrine pancreas.

Since the works of WHARTON (1932) and THIEL (1954), the portal connections now called the "insulo-acinar portal system" (FUJITA, 1973; FUJITA and MURAKAMI, 1973)
between the capillary network in the islets and that in the acinar tissue have been confirmed by light microscopy of dye-injected specimens or by scanning electron microscopy of corrosion casts in a variety of animals (horse: Fujita, 1973; monkey: Fujita and Murakami, 1973; dog, rat, rabbit: Fujita, Yanatori and Murakami, 1976; cat: Syed Ali, 1984). The portal connections between the islets and the excretory ducts, and those between the exocrine acini and the excretory ducts in the pancreas, have also been described in the rat and rabbit (Ohtani and Fujita, 1980; Lifson and Lassa, 1981).

Intravital microscopy of the pancreas in living animals has evidenced that blood flows from the islet through the insulo-acinar portal vessels to the exocrine acini (mouse: McCuskey and Chapman, 1969; rabbit: Fraser and Henderson, 1980; rat: Ohtani, 1983), and that the pancreatic ducts receive blood from the capillary beds of islets and exocrine acini as well as from arterioles (Ohtani, 1983). Thus, islet secretions can be transported in high concentrations to the exocrine pancreas, including the ducts. Physiological experiments show that pancreatic enzyme output is regulated by insular secretions (reviewed by Henderson et al., 1981; Henderson, 1983).

Recently Bonner-Weir and Orci (1982), based on their SEM of vascular corrosion casts, and Nishino (1984), referring to his intravital microscopy of living animals, claimed that in the rat, only some of the efferent vessels of the islet contribute to the formation of the insulo-acinar portal system. Our previous studies by intravital microscopy in conjunction with the SEM of vascular corrosion casts also showed that the rat islets often possessed both emissary veins (so designated by Fujita and Ohtani, 1984) leading into the systemic veins, and the insulo-acinar (and insulo-ductular) portal vessels (Ohtani, 1983). Although it is known that there are substantial interspecies differences in the microvascular organization of the pancreas (reviewed by Ohtani et al., 1983), how much the patterns of microcirculation in this organ differ between species is still obscure. Accordingly, it remains to be studied whether rodents fit the generalized microcirculatory pattern of the pancreas. Furthermore, few reports are available, as far as we know, as to how far or to what extent the blood leaving the islets can reach the exocrine pancreas through the insulo-acinar portal vessels.

This paper, therefore, aims at re-examination of the microvascular organization of the pancreas, especially of the islets in the rat and the rabbit by SEM of vascular corrosion casts, light microscopy of India ink-injected/cleared materials, and intravital microscopy of the living pancreas.

MATERIALS AND METHODS

The animals used were healthy adult Wistar rats weighing 150-250 g and Japanese rabbits (2.5-4 kg BW) of either sex, fed combined solid food for laboratory animals (MF for rat; ARC4 for rabbits, Oriental East Co., Tokyo) with free access to water.

SEM of vascular corrosion casts

Vascular corrosion casts of the pancreas of ten rats and two rabbits were made with injection of semipolymerized methylmethacrylate (Mercox, Japan Vilene Hospital, Tokyo) according to the method by Murakami (1971).

SEM observation, following microdissection of the casts under a stereo-light microscope, was repeated until the deeper structures were satisfactorily observed (Fujita and Murakami, 1973). Stereo-pairs of SEM images were frequently taken with a tilt separation of 4-6 degrees.
Some casts fixed on the specimen-holders were embedded in ice and a superficial part of about 0.1-0.2 mm in thickness was cut out with a specially designed microtome. They were then air-dried, coated with heavy metal and observed in an SEM. This series of procedures was repeated several times until three-dimensional vascular connections in and around the islets were completely analysed.

One hundred vascular casts of islets with their connecting vessels in the rat and fifty in the rabbit were randomly chosen from various regions of the pancreas and examined under a SEM (JSM-U3, JEOL, Tokyo) with an accelerating voltage of 5 kV.

**Intravital microscopy**

The method employed here is basically the same as that described by GANNON et al. (1982), who studied the rat stomach, and as the technique applied to the rat pancreas in our previous paper (OHTANI, 1983).

**Animals and anesthesia:** Fifty rats and two rabbits were anesthetised by an injection of sodium pentobarbital (Nembutal; 50 mg/100g BW; Abbot Lab., North Chicago) either intravenously (in the rabbit) or intraperitoneally (in the rat).

**Preparation of the pancreas:** The abdomen was shaved and a small incision of 2–3 cm was made in the subcostal region. An appropriately bent image conduit was inserted into the abdominal cavity; the conduit was placed dorsal to the pancreas. The pancreas to be observed was stabilized between the end of the conduit and a 20 mm diameter plexiglass disc which had a 10 mm diameter central aperture covered with a clear plastic film (Saran Wrap, Asahi Kasei, Tokyo). Both the image conduit and stabilizing disc were fixed on the plexiglass plate on which the animal was placed. All other abdominal organs exposed were irrigated with saline (38°C) and covered with a plastic film.

**Light microscopy and recording:** The animal preparation on the plexiglass plate was attached to the stage drives on either an Olympus BH microscope or a specially designed intravital microscope. The pancreas preparation was observed by either transillumination with a Nikon fiber optic light source or an Olympus incident fluorescent illuminator plus 100 W power supply (Olympus, USH-102D). The optical image was projected to a television camera (Ikegami CTC-2100 or Hitachi DK-5001) observed on a TV monitor (PM-950, Ikegami Tsushinki, or -14R, Victor, Tokyo) and recorded on video tape with a video cassette recorder (SLO-333 or SL-F7, Sony, Tokyo). Using this technique, fifty islets in the rat and twenty in the rabbit were examined. In order to improve the visibility of blood vessels and to confirm the flow pattern, a small amount of India ink was injected intravenously in some of the animals.

**Fluorescent tracer:** In order to examine the flow pattern within the islets, 5% Uranine (Katayama Chemical, Osaka) in 0.9% saline was injected through a cannula inserted retrogradely into the aorta so that its tip was positioned near the branching point of the celiac artery.

**Light microscopy of India ink-injected/cleared pancreas**

After the intravital microscopic observations, certain animals were arterially injected with India ink. The pancreas then was fixed in situ by irrigation of 10% formalin and removed to be immersed in the same fixative. It was dehydrated in a graded series of ethanol, cleared in methyl salicylate and observed under a stereo-light microscope.
RESULTS

The results showed no evidence of any sex-related differences.

SEM of vascular corrosion casts

*Microvascular pattern common to the rabbit and rat*

Lobules of various sizes and shapes were obvious upon gross inspection of the vascular corrosion casts of the pancreas. The interlobular artery ran with its venous partner, sending off intralobular arteries and arterioles.

The intralobular (terminal) arterioles fell into three categories according to their destinations: 1) vasa afferentia of the islets, 2) acinar arterioles which directly divided into capillaries surrounding acini, 3) arterioles supplying the duct system by forming the periductular (and periductal) capillary plexus (Fig. 1). The vasa afferentia of the islets accounted for about 10–20% of the total terminal arterioles in both the rat and

![Image](image_url)

**Fig. 1.** A scanning electron micrograph of a vascular corrosion cast of the rat pancreas. Three types of intralobular arterioles can be recognized: an insular arteriole (arrowhead) supplying the capillary glomerulus of the islet of Langerhans (L); acinar arterioles (aa) branching out into capillaries around acini; and arterioles (ad) that supply the pancreatic ductule (tinged white) by forming the periductular capillary plexus. The capillaries of the islet are seen gathered into an emissary veins (ev). The capillary vasa efferentia of the islet branching out into the acinar capillaries, i.e., the insulo-acinar portal vessels (e) and those joining the periductular plexus (ed) can also be recognized. v Intralobular venule, A, V interlobular artery and vein. ×190
An apparent characteristic of the microcirculation was that the acinar arterioles preferentially supplied the exocrine pancreas far removed from the islets. Usually one or two, but sometimes more vasa afferentia supplied individual islets (Fig. 2-8). After reaching an islet, the vas afferens divided into swollen sinusoidal capillaries (approximately 12 μm in caliber) running along the cortical portion of the islet. The cortical sinusoidal capillaries frequently took U-shaped turns and gave off secondary branches entering the core of the islet (Fig. 2-8).

Within the islet existed a few capillaries of small caliber, some of which arose deep in the core of the islet and contributed to the vasa efferentia of the islet (Fig. 5, 6). The vasa efferentia radiated from the islet and connected either with the capillary network in the exocrine pancreas, including the pancreatic duct system, or with nearby veins (see below).

The capillaries in the exocrine portion were much smaller (approximately 7 μm in caliber) than those in the islet, and formed complicated networks. These capillaries in the exocrine pancreas were gathered into venules which in turn collected into larger
intralobular venules (Fig. 1). The intralobular venules took winding courses and tended to run the periphery of the lobule, in contrast to the intralobular arterioles which took rather straight courses and tended to run along the center of the lobule.

Microvasculature of the rabbit islet

The rabbit islet ranged from 80 to 300 \( \mu m \) in diameter. Some vasa efferentia were large and ran for a distance before they branched out into capillaries in the exocrine pancreas (Fig. 2, 3, 5). Only two out of fifty islets possessed emissary venules (approximately 15–20 \( \mu m \) in caliber) that converged into a small vein draining into the systemic circulation (Fig. 4, 6). Emissary vessels of capillary appearance could be traced as far as 200–250 \( \mu m \) away from the islet before draining into venules, although the distance varied with the size and location of the islet.
Smaller islets were usually located deep in the lobule, surrounded by the exocrine pancreas. The microvascular organization of such islets was similar to that of the rabbit islet described above, except that the rat islets more frequently possessed one or two emissary veins leading directly into the systemic circulation than did the rabbit islets (Fig. 1, 6).

The rat pancreas had large ovoid or rod-like islets; the largest ones measuring about 200 × 800 μm. They were usually located in the superficial part of the lobule, or adjacent to the pancreatic duct or ductule (Fig. 1). On the surface of such large islets, the sinusoidal capillaries converged into venules which collected into one or two (or more) emissary veins leading into nearby larger veins (Fig. 7). Such large islets also possessed the insulo-acinar and/or insulo-ductular portal vessels (Fig. 1, 7).

Rat islets bearing the emissary veins accounted for approximately 60% of the total

**Microvasculature of the rat islet**

Smaller islets were usually located deep in the lobule, surrounded by the exocrine pancreas. The microvascular organization of such islets was similar to that of the rabbit islet described above, except that the rat islets more frequently possessed one or two emissary veins leading directly into the systemic circulation than did the rabbit islets (Fig. 1, 6).

The rat pancreas had large ovoid or rod-like islets; the largest ones measuring about 200 × 800 μm. They were usually located in the superficial part of the lobule, or adjacent to the pancreatic duct or ductule (Fig. 1). On the surface of such large islets, the sinusoidal capillaries converged into venules which collected into one or two (or more) emissary veins leading into nearby larger veins (Fig. 7). Such large islets also possessed the insulo-acinar and/or insulo-ductular portal vessels (Fig. 1, 7).

Rat islets bearing the emissary veins accounted for approximately 60% of the total
islets examined, while all the other islets possessed exclusively the insulo-acinar portal vessels.

Surface morphology of the casts suggestive of sphincters

Marked constrictions were frequently observed at the branching point of the intralobular arterioles (Fig. 2). The vas afferens frequently showed circularly oriented constrictions (Fig. 2). A cast of the vas efferens of the islet sometimes showed marked constrictions at the islet-exocrine border (Fig. 2). It was also at the junction of an emissary vein of the islet with a larger vein that a distinct constriction was sometimes found (Fig. 7).

Fig. 5. A stereo-pair of part of Figure 3. The insular arteriole (a) is divided into capillaries that run tortuously in the superficial part of the islet (L), giving off secondary capillary branches into the core of the islet en route. e Insulo-acinar portal vessel. × 195

Fig. 6. A stereo-pair of part of Figure 4. The sinusoidal capillaries (c') arising from the insular arteriole (a) run superficially along the islet, giving off secondary capillaries (c") which penetrate into the core and there turn towards the cortex to be continuous with vasa efferentia (e). ev Emissary venule. × 195
Light microscopy of India ink injected/cleared pancreas

Whole mount preparations of India ink injected and cleared pancreas in the rabbit showed that the intralobular arteries ran along the center of the lobule, and gave off the vasa afferentia of the islets and the acinar arterioles (Fig. 8). Each lobule appeared to be made up of subdivisions (or sublobules), each of which consisted of a centrally located capillary glomerulus of the islet and the surrounding capillary network of the exocrine tissue.

Intravital microscopy

Examination with an intravital microscope showed that 26 of 50 islets in the rat possessed emissary veins. All the islets observed emitted capillary vasa efferentia...
through which blood was confirmed to flow from the islet to the exocrine pancreas.

Arterial injection of a fluorescent tracer or India ink during observation under the intravital microscope revealed that blood reaching the islet first perfused the cortical area of the islet, then the core portion and exited the islet through the insulo-acinar portal vessels and/or emissary veins (Fig. 9).

In the rabbit no islet possessing an emissary vein could be found under the intravital microscope.

In both species, the exocrine areas within the range of about 200 μm away from the islet were observed to be irrigated by blood from the islet.

DISCUSSION

Arterial supply to the endocrine pancreas

The present study has demonstrated in the rat and rabbit that about 10–20% of the terminal arterioles of the pancreas directly supplies the islets, while the remainder supplies the exocrine pancreas. Lifson and associates (1980) who perfused the rabbit pancreas with microspheres as markers, reported similar results in that islets received about 15–20% of the blood passing the organ. Since the islets constitute 1–2% of the total mass of the gland, the islet flow in terms of tissue weight must be about ten times that of the exocrine pancreas (Henderson, 1983).

Intrainsular microcirculation

The microvascular organization within the islet in the rat and rabbit demonstrated in this study is consistent with that of our previous studies (Fujita, Yanatori and Murakami, 1976; Ohtani and Fujita, 1980, 1981). In these animals, the vasa afferentia
of the islet are divided into sinusoidal capillaries in the cortical portion of the islet where A and D cells are located, and their secondary branches penetrate deep into the islet core occupied by B cells. In the horse and the monkey, however, the vas afferens enters the core which is occupied by A and D cells in these species, and breaks up into sinusoidal capillaries which radiate to the cortex consisting of B cells (Fujita, 1973; Fujita and Murakami, 1973). The interspecies differences of the islet microvasculature suggests that blood flows from A and D cell area to the B cell area (Fujita, 1973), which is consistent with the widely accepted view that insulin release from B cells is stimulated by glucagon released from A cells and inhibited by somatostatin from D cells. Recently Van Schravendijk et al. (1985) reported the existence of a pool of high affinity glucagon receptors on purified B cells.

Some authors (Bonner-Weir and Orci, 1982; Unger, 1983), however, envisage that blood reaches the center of the rat islet first, so that blood stimulates B cells first and A and D cells second. As discussed above, our corrosion casts revealed that the vas afferens first supplies a quite substantial area of the cortex by forming highly tortuous sinusoidal capillaries whose branches enter the core, while the vasa efferentia arise deep in the islet. Furthermore, intravital microscopy using a fluorescent tracer revealed that blood flows from the cortex (A and D cell area) to the core of the islet (B
Fig. 10. A schematic presentation of microcirculatory routes through an islet. This diagram also shows a microcirculatory unit of the pancreas (a: rat, b: rabbit). An islet is located in the center of the unit. The arteries (A) and veins (V) position at the periphery of the unit. An insular arteriole (a) forms a capillary glomerulus in the islet which in turn issues numerous vasa efferentia (e insulo-acinar portal vessels) branching out into the periacinar capillary network. The rat islet possesses emissary veins (ev) leading into larger veins, in addition to the insulo-acinar portal vessels, while one in the rabbit has few emissary veins. Arrows indicate directions of blood flow.
cell area) and then flows into the vasa efferentia. Nishino (1984) also demonstrated a similar intrainsular flow pattern in the rat by intravital microscopy with dye injection. Thus, it seems evident that arterial blood flows from the islet cortex to the core in the rat and rabbit. The interstitial fluid within the islet will course following the blood flow.

**Insulo-acinar portal system and emissary veins of the islet**

The present SEM observations of vascular corrosion casts have again confirmed the existence of an insulo-acinar portal system in the rat and rabbit (Fujita, Yanatori and Murakami, 1976; Ohtani and Fujita, 1980, 1981). Intravital microscopy of pancreatic microcirculation has also confirmed that blood leaving the islet flows through the insulo-acinar portal vessels into the exocrine pancreas as reported by McCuskey and Chapman (1969) and Fraser and Henderson (1980) and by one of the authors (Ohtani, 1983).

The present study, however, revises our previous view by demonstrating the occurrence of direct venous routes draining the insular blood. It is estimated by SEM of vascular corrosion casts and intravital microscopy of microcirculation that, in the rat, about half of the blood leaving the islets flows through the emissary veins of the islet into the systemic veins. Emissary veins in the rat have been reported by Bonner-Weir and Orci (1982) in their SEM study of vascular corrosion casts and by Nishino (1984) in his microscopic observation of the pancreatic microcirculation in situ, with and without intravascular dye injection. As Nishino (1984) noted, the emissary veins are mainly found in large islets positioned in the superficial part of the lobule or in the interlobular spaces. Most of the smaller islets in the rat are located within the lobule and most of their vasa efferentia are insulo-acinar portal vessels. Thus, the existence of the emissary veins of the islet does not essentially reduce the significance of the insulo-acinar portal system, since many of the exocrine acini are perfused with the blood containing insular secretions in high concentrations.

In the rabbit pancreas, however, islets preferentially possess the insulo-acinar portal vessels with only occasional small emissary veins. In the cat, islets with an emissary vein are found only exceptionally (Syed Ali, 1984). Our preliminary studies on the dog pancreas have also shown that dog islets exclusively possess insulo-acinar portal vessels with very rare emissary veins if any. In this connection, it is evident that well-developed emissary veins of the islet are characteristic of the rat, though they are few in other animals hitherto examined.

Intravital microscopy has shown that blood flows through the insulo-acinar portal vessels into the exocrine capillaries within the range of 250 μm from the islet before they collect into venules. SEM of corrosion casts and light microscopy of India ink injected and cleared rabbit pancreas have shown that the pancreatic lobule can be divided into subdivisions or microcirculatory units, the islet being located in the center of each subdivision. The periphery of the unit also receives a direct supply of the intralobular arterioles. Such an arrangement is much clearer in the dog, where the pancreatic lobules are made up of roughly spherical microcirculatory units, and the center of each unit is occupied strategically by an islet radiating the insulo-acinar portal vessels (details to be reported elsewhere). Thus, the insulo-acinar portal system simply forms a microcirculatory unit of the pancreas. The unit may be designated as the primary lobule, as proposed by Yaginuma et al. (1981) in their reconstruction of serial sections of human pancreas.

According to present and previous studies of our research group (Fujita, 1973;
FUJITA and MURAKAMI, 1973; FUJITA, YANATORI and MURAKAMI, 1976; OHTANI and FUJITA, 1980, 1981; OHTANI, 1983; OHTANI et al., 1983), we can now summarize the islet microcirculation as shown in Figure 10.

The functional significance of the insulo-acinar portal system has been extensively reviewed (FUJITA and KOBAYASHI, 1979; HENDERSON et al., 1981; HENDERSON, 1983). Recent physiological experiments have indicated that insulin secretion has a direct effect on pancreatic enzyme output. This supports the hypothesis that the insulo-acinar portal system plays an important role in the regulation of acinar cell function (TRIMBLE et al., 1985).

The emissary venules, on the other hand, are considered to provide a short passage-way for the blood in the islets to reach the general circulation. As NISHINO (1984) suggested, they seem a device to quickly convey insular hormones to the whole body. They might play a particularly important role in the case of emergencies like hyperglycemia and hypoglycemia.

Possible occurrence of the sphincters

The structures that are responsible for regulating the islet blood flow remain unknown. Casts of the vas afferens of the islets frequently show circular constrictions suggesting the existence of smooth muscles which may be involved in regulating arterial blood supply to the islet. Marked constriction at the commencement of the vas afferens of the islet also supports the idea that a sphincter here might regulate the islet blood flow.

Occasional constrictions of the cast of the insulo-acinar portal vessels at the islet-exocrine border further indicates the locations of sphincter-like structures. SYED ALI (1984) suggested that the pericytes surrounding the capillaries at the islet-exocrine border might serve as regulators of the irrigation of certain regions of the exocrine pancreas.

Constrictions of the cast at the junctions of the emissary veins with larger veins also suggest the presence of sphincters. RHODIN (1968) described sphincters in the postcapillary venules in the rabbit mesentery. If the sphincters do exist in the emissary veins (or venules) of the islet, they likely serve as switchovers of the blood flow between the routes of insulo-acinar portal vessels into the exocrine pancreas and the short circuit of the venous drainage, since a majority of the rat islets possess both the insulo-acinar portal vessels and the emissary veins.

REFERENCES


